REMARKS/ARGUMENTS

Claims 1-69 have been subjected to substantive examination. Claims 1, 5, 6, 23, 24, 25, and 40 have been amended. Support for the amendments can be found in the specification as set forth in greater detail below. Therefore, no new matter has been added. Applicants respectfully request the Examiner reconsider the pending claims in light of the remarks below.

Double Patenting

Claims 40 and 60-69 have been provisionally rejected by the Examiner on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-26 of co-pending Application No. 10/992,154. The Examiner asserts that although the conflicting claims are not identical, they are not patentably distinct from each other because they commonly comprise methods of using cross-flow membrane filtration to enrich recirculating solutions in leukocytes so as to culture stem cell populations. According to the Examiner, this is a provisional obvious-type double patenting rejection because the conflicting claims have not in fact been patented.

Applicants respectfully disagree with the rejection and do not acquiesce to the Examiner's reasoning to support such a rejection. Applicants further direct the Examiner to the amendment filed in Application No. 10/992,154 wherein claim 1 has been amended to recite a step of *pretreating the sample to induce cell shrinkage of cell populations with essentially the same size as the stem cells*. The present application does not teach or suggest such a step. Thus, Applicants respectfully submit that the provisional double patenting rejection of claims 40 and 60-69 is now moot and further request that the provisional rejection on the grounds of nonstatutory obviousness-type double patenting be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 1-22 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. According to the Examiner, in claim 1, "the sample..." lacks antecedent basis or is inconsistent with "cell population."

Applicants respectfully disagree with the rejection. However, to more clearly define the presently claimed device and further expedite prosecution of the application, claim 1 has been amended to read "the cell population comprising a sample of blood constituents comprising leukocytes". Support for this amendment can be found throughout the specification and, for example, at page 11, line 24 though page 12, line 22. Dependent claims 5 and 6 have also been amended to be consistent with the wording now used in claim 1. Applicants also note that independent claims 23, 24, 25 and 40 have been amended to make them consistent with the language used in amended claim 1. For the reasons above, Applicants respectfully request that the Examiner reconsider and withdraw the rejection of claims 1-22 under 35 U.S.C. § 112, second paragraph.

Rejections under 35 U.S.C. § 102(b)

Claims 1-5, 12, 13, 23, 25 and 27-29 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Brody *et al.* (U.S. Patent No. 5,922,210, herein referred to as "Brody"). The Examiner asserts that Brody discloses a tangential flow filtration device comprising a remover unit having a cross-flow chamber 4/6, a filtrate chamber 3, a filter 5, an inlet 1 to direct flow parallel to the filter, a centrally disposed outlet 7 opposite/facing the filter surface, the filter having a possible range or pore sizes overlapping 1-10 microns, such that the flow across the filter enriches a sample population of blood containing either red blood cells, white blood cells (leukocytes), or both.

Applicants respectfully disagree with the Examiner and traverse below. Applicants submit that Brody fails to teach each and every element of the device of claim 1. For

instance, Applicants cannot find any teaching or suggestion of an *outlet centrally disposed* in a portion of the cross-flow chamber *opposite the filter surface*, as presently claimed. As shown in Fig. 1, Brody teaches a microfilter with a cross-flow chamber wherein the outlet (or, feed exit, 2) runs parallel to a barrier channel (depicted in Fig. 1) and is disposed at the end of a feed flow channel, which is configured to extend past the barrier channel region. The outlet (7) noted by the Examiner to be the same as the outlet (7) as set forth in the present application is part of the filtrate chamber not the cross-flow chamber as set forth in the present claims. Also, Brody describes a filtration device directed towards collecting cell-free plasma by filtering out particulates in blood. The reference provides no teaching or suggestion on how to separate or enrich a sample comprising blood constituents including various blood cells for leukocytes; all of the constituents except for plasma are sent to the feed exit (2). Brody fails to teach or suggest this aspect. For these reasons above, Brody cannot anticipate claim 1 and its respective dependent claims 2-5, 12, and 13.

The Examiner, further alleges that Brody discloses the following for claim 2: a means for providing predetermined input rate and filtration rate. In addition, the Examiner states "the filtration rate optionally the input rate." Applicants are unclear on the Examiner's assertion and request clarification should the Examiner find it is necessary after reconsideration of the rejection. In view of the comments set forth above and below, however, Applicants believe the Brody does not teach either the device or the method as recited in the present claims. Brody does not teach an outlet centrally disposed in a portion of the cross-flow chamber opposite the filter surface. As to claims 3 and 4, the Examiner submits that Brody teaches a pore size of about 3-5 microns and, for claim 5, a blood source. Regarding claims 12 and 13, the Examiner asserts that Brody discloses handling/separation/processing of the fluids both upstream and downstream for further processing of the fluids.

Again, Applicants disagree with and traverse the rejection. Applicants submit that the pending claims including claims 2-5, 12, and 13 are not anticipated by Brody. Specifically, Brody fails to teach or suggest an *outlet centrally disposed* in a portion of the cross-flow chamber *opposite the filter surface*. Brody also does not *enrich the sample of blood*

constituents for leukocytes. In addition, regarding claim 2, Applicants cannot find any teaching or suggestion of where the filtration rate is limited *to less than the unopposed (i.e. open tube) filtration rate for the filter*.

Accordingly, Applicants respectfully submit that Brody fails to anticipate the presently claimed device and the Examiner is respectfully requested to reconsider and withdraw the rejection of claims 1 and dependent claims 1-5, 12, 13 under 35 U.S.C. § 102(b) as being anticipated.

For independent claims 23-25, the Examiner alleges that Brody also discloses, the device being used in sampling and analysis, the inlet being disposed above the filter (citing "applying of gravitational forces") and the outlet 7 being optionally disposed to the upper portion of cross-flow chamber. According to the Examiner, use of gravitational forces apparently implies flow of feed liquid being above or across the top of the membrane filter. The Examiner further asserts that the flow rate may be increased or decreased through the filter because the reference allegedly concerns starting and stopping of pressure applying to start and stop flow, backflow through the filter at times at about half the rate of pressure, and flow during filtration mode. The Examiner submits that the filter appears to be horizontal with the top plane of chamber. Also, the Examiner submits that Brody discloses blood, plasma, and red and white blood cells ("erythrocytes" and "leukocytes") for claims 27-29. From this alleged disclosure, the Examiner believes that platelets are also inherently present and any population of leukocytes necessarily contains some amount of monocytes. Finally, for claim 33, the Examiner asserts that Brody discloses "electroendoosmotic forces" together with "surface tension forces" overlap recites applying of tangential forces.

Applicants disagree with the Examiner's rejection and reasoning to support the rejection. Applicants respectfully traverse and submit that claims 23-25 are not anticipated by Brody. Brody fails to teach or suggest an *outlet centrally disposed in an upper portion of the chamber*. As shown in Fig. 1, Brody instead teaches a microfilter with an outlet (or, feed exit, 2) that runs parallel to the barrier channel (depicted in Fig. 2) and is disposed at the end of a feed

flow channel, which is configured to extend past the barrier channel region. The outlet (7) identified by the Examiner is not part of the cross-flow chamber, but is instead a part of the filtrate chamber. Therefore, Brody cannot teach or suggest the device of claim 23.

With regards to each claim 23 and 25, Brody fails to teach or suggest *enrichment of leukocytes from a sample of blood constituents*. Brody is directed to separating plasma from other blood particulates, such as red and white blood cells; the reference is not directed to, and is incapable of, enriching leukocytes. In fact, column 4, lines 21-24 of Brody states "[t]he desired output of the microfilters of this invention is a quantity of liquid from which particles have been removed, rather than the particles themselves." Thus, Brody doesn't even attempt to separate among the various particulates, *i.e.*, cells, let alone enrich the sample for leukocytes. For the reasons set forth above, Applicants submit that claims 23 and 25 and their respective dependent claims including claims 27-29 are not anticipated.

Applicants note that the Examiner did not list claim 24 in the rejection beginning on page 3 of the Office Action mailed April 21, 2008. However, on page 4 in the same rejection, the Examiner states "For independent claims 23-25." In view of the Examiners rejection to claim 24 under 35 U.S.C. § 103(a), which states that the claim differs from Brody by requiring a predetermined concentration of blood cells, Applicants interpret the § 102(b) rejection to include only claims 23 and 25 and not claim 24. Applicants request that the Examiner clarify any misunderstandings, if such clarification is deemed necessary. Yet, in view of this interpretation and the comments above, Applicants respectfully request that the Examiner reconsider and withdraw the rejection of claims 1-5, 12, 13, 23, 25, and 27-29 under 35 U.S.C. § 102(b) as being anticipated by Brody.

Rejections under 35 U.S.C. § 103(a)

Claims 24, 31, and 32 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Brody (*supra*). According to the Examiner, claim 24 differs in explicitly requiring predetermined concentration of blood cells to about 10 (7th-10th power)/milliliter and

filtration rates of 1/5 to 1/100th the predetermined input rate. The Examiner asserts, however, that Brody recites applying multiple positive and negative pressure to the flow, which obviously encompasses any range of increasing or decreasing filtration rates. The Examiner further asserts that concentrations of blood cells are obviously a factor of both selection of blood source(s) and membrane filter pore sizes utilized. The Examiner also submits that claims 31 and 32 differ in explicitly requiring an enriched cell population of at least about 20% or at least about 60% leukocytes. But, according to the Examiner, proportion of leukocytes in cell population would obviously vary with selection of blood sources and membrane filter pore sizes.

Applicants respectfully disagree with the Examiner's rejection and the reasoning to support such a rejection. As an initial matter, section 2143 of the MPEP states, "[t]he key to supporting any rejection under 35 U.S.C. § 103 is the clear articulation of the reason(s) why the claimed invention would have been obvious." The Examiner instead merely makes conclusory statements and provides no articulation or reasoning why the alleged differences in claim 24 would have been obvious. Thus, Applicants respectfully submit that the rejection fails for this reason alone. However, as already discussed, Brody fails to teach or suggest each and every element of the present claims. For example, Brody does not teach an outlet centrally disposed in a portion of the *cross-flow chamber* opposite the filter surface, as presently claimed. Instead, as shown in Fig. 1, Brody teaches a microfilter with a feed exit (2) that runs parallel to the barrier channel (depicted in Fig. 2) and is disposed at the end of a feed flow channel, which is configured to extend past the barrier channel region. The Examiner has cited the outlet (7) as being so placed. As above, the outlet (7) is not as part of the cross-flow chamber, but instead is a part of the filtrate chamber. Therefore, Brody fails to teach an outlet centrally disposed in a portion of the *cross-flow chamber* opposite the filter surface.

In addition, while Brody does mention red and white blood cells, the filtration is directed toward collecting cell-free plasma by filtering out particulates in blood. The reference provides no teaching or suggestion as to how to separate or enrich the other particulates from the blood; all of the constituents except for plasma are sent to the feed exit (7). In contrast, the presently claimed device enriches the cell population comprising a sample of blood constituents

for leukocytes, *i.e.*, leukocytes are enriched over other particulates because red blood cells and plasma, for example, are selectively removed from the sample. Brody fails to teach or suggest this aspect. Furthermore, the predetermined filtration rates and blood cell concentrations as presently claimed would not have been obvious to one of ordinary skill at the time of invention. Specifically, the skilled artisan could not have predicted what concentrations or flow rates would result in a sample that is enriched for leukocytes based on the teachings of Brody that are directed to separating plasma from a blood sample and the Examiner has provided no reasoning as to why such a result could be predicted. For the reasons above, Applicants respectfully request that the Examiner reconsider and withdraw the rejections to claims 24, 31, and 32 under 35 U.S.C. § 103(a) as being unpatentable over Brody.

Claims 6-10, 26, 30, 40-49 and 60 stand rejected under 35. U.S.C. § 103(a) as being unpatentable over Brody (*supra*) in view of Castino *et al.* (U.S. Patent No. 4,420,398, previously of record, herein referred to as "Castino"). The Examiner asserts that Castino teaches a method and system for separation leukocytes from blood sources originally obtained as whole blood samples from human patients or donors. The Examiner submits that the blood is introduced into cross-flow membrane-containing remover units 11 and 21 through respective inlets where leukocytes are selectively removed from other blood components and constituents to form cell populations that are enriched for leukocytes. The Examiner alleges that the respective retentate populations are continuously recirculated between cross flow membrane 24 in chamber 21 and cell recovery until ("CPAS reservoir") to cell populations enriched in forms of leukocytes ("production broth" and CPAS reservoir), respectively. According to the Examiner, Castino also teaches the following: the cell populations being prepared by samples upstream filtration or leukopheresis, the blood constituents naturally contain plasma, platelets, erythrocytes, etc. and the recycling of stream volumes may be carried out indefinitely (as allegedly required in claims including claims 42-47). The Examiner submits that Castino further discloses a means for heating to controlled temperature and control of filtration flow rates, filter pore size of about 3-5 microns or adapted to retain leukocytes, and blood sources, recovery unit and crossflow filter being in a loop format and connected by inlets and outlets to the units, and a means for culturing.

Claims 6, 26 and 42 and claims dependent therefrom are alleged by the Examiner to differ in requiring a leukophoresis device to be the blood source. Brody is asserted by the Examiner to disclose previous handling and separation of the blood source. As such, the Examiner asserts that it would be obvious to one of ordinary skill in the art to have employed the leukophoresis device of Castino in the Brody system in order to control and tailor the amount of leukocytes in the permeate or retained/enriched fluid fractions.

Claims 7-10 and 40-48 are alleged to differ in requiring recirculating of blood sample fluid through the unit involving a recovery unit communicating the cross-flow chamber in loop/recycling format, by communication of the inlets and outlets of the chamber and unit. The Examiner believes that it would have been obvious to have employed the recovery unit and recycling loop of Castino in the Brody device or method, to result in achieving much greater purity of blood components and constituents in fluid fractions.

Claim 30 is alleged by the Examiner to require repeating of steps a plurality of times to form enriched populations of leukocytes. The Examiner believes that Castino infers the recirculating process being continuous or constant, hence cell populations pass across the cross-flow membrane any number of times.

Applicants respectfully disagree with the rejection and reasoning and reiterate, as set forth above, Brody fails to teach or suggest multiple elements of the present claims. Brody does not teach an outlet centrally disposed in a portion of the *cross-flow chamber* opposite the filter surface, as presently claimed. Instead, as shown in Fig. 1, Brody teaches a microfilter with an outlet (or, feed exit (2)) that runs parallel to the barrier channel (depicted in Fig. 2) and is disposed at the end of a feed flow channel, which is configured to extend past the barrier channel region. In addition, the Examiner has pointed to the outlet (7) as being the outlet centrally disposed in a portion of the cross-flow chamber. As set forth above, the outlet (7) is not in a portion of the cross-flow chamber, but instead is disposed in a portion of the filtrate chamber.

Further, while Brody does mention red and white blood cells, the filtration is directed towards collecting cell-free plasma by filtering out particulates in blood. The reference provides no teaching or suggesting on how to separate or enrich the other particulates in the blood; all of the constituents except for plasma are sent to the feed exit. In contrast, the presently claimed device enriches the sample of blood constituents for leukocytes, *i.e.*, leukocytes are enriched over other particulates because red blood cells and plasma, for example, are selectively removed from the sample. Brody fails to teach or suggest this aspect.

Castino fails to provide any of the missing elements of Brody. Applicants direct the Examiner's attention to Applicants' previous arguments, *e.g.*, on pages 14-15 in the response filed on December 28, 2007, which further point out why Castino fails to render the claims obvious. In addition, while Castino uses cross-flow membrane filtration, the reference is directed to extracting cell produced antiviral substances (CPAS), which can be, for example, an interferon and other biomolecules that are in the weight ranges of tens to hundreds of thousands Dalton. Castino teaches that leukocytes can be used to produce the CPAS, but the leukocytes are not of interest with respect to the filtration; they merely serve as producers of CPAS. Therefore, while Castino may teach enrichment of the molecule designated CPAS, the reference fails to teach the enrichment of a cell population in any cell, much less leukocytes. Furthermore, Castino provides no schematic configurations of the filtration equipment; thus, it cannot teach or suggest any organization of the various parts of a filtration device. In particular, a device as presently claimed.

Applicants respectfully request the Examiner reconsider and withdraw the rejection of claims 6-10, 26, 30, 40-49 and 60 under 35. U.S.C. § 103(a) as being unpatentable over Brody (*supra*) in view of Castino.

Claim 11 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Brody (*supra*) in view of Castino (*supra*) as applied to claims 1-10 above, and further in view of Raehse *et al.* (US 4,751,003) or Harm (US 4,722,902). Specifically, the Examiner alleges that claim 11 additionally requires the cross-flow chamber to be cylindrical. Both Raehse and Harm

are cited by the Examiner as showing cylindrical filter membranes for crossflow separation of blood components. The Examiner believes that it would have been obvious to have utilized the cylindrical filter membranes of Raehse or Harm also to enhance separation efficiency, since crossflow in such blood separation has been shown to result in near 100% separation rates (Abstract of Raehse).

Applicants must again respectfully disagree with the rejection of the Examiner and reiterate that as explained above Brody fails to teach or suggest multiple elements of the present claims. Brody does not teach an outlet centrally disposed in a portion of the *cross-flow chamber* opposite the filter surface, as presently claimed. Instead, as shown in Fig. 1, Brody teaches a microfilter with an outlet (or, feed exit (2)) that runs parallel to the barrier channel (depicted in Fig. 2) and is disposed at the end of a feed flow channel, which is configured to extend past the barrier channel region. The Examiner cited to the outlet (7) as being centrally disposed in a portion of the cross-flow chamber opposite the filter surface, but as pointed out above, the outlet (7) is part of the filtration chamber and not the cross-flow chamber. Also, while Brody does mention red and white blood cells, the filtration is directed towards collecting cell-free plasma by filtering out particulates in blood. The reference provides no teaching or suggestion on how to separate or enrich the other particulates in the blood; all of the constituents except for plasma are sent to the feed exit. In contrast, the presently claimed device *enriches the sample of blood constituents for leukocytes*, i.e., leukocytes are enriched over other particulates because red blood cells and plasma, for example, are selectively removed from the sample. Brody fails to teach or suggest this aspect.

Castino fails to provide the missing elements of Brody. Applicants again respectfully direct the Examiner's attention to Applicants' previous arguments, *e.g.*, on pages 14-15 in the response filed on December 28, 2007, which point out why Castino fails to render the claims obvious. In addition, while Castino uses cross-flow membrane filtration, the reference is directed to extracting cell produced antiviral substances (CPAS), which can be, for example, an interferon or other biomolecules that are in the weight ranges of tens to hundreds of thousands Dalton. As the reference indicates, leukocytes can be used to produce the CPAS, but the

leukocytes are not of interest with respect to the filtration; they merely serve as producers. Therefore, while Castino may teach enrichment of cell produced substances, the reference fails to teach or suggest any device or method that might be used to separate cells. Furthermore, Castino provides no schematic configurations of the filtration equipment; thus, it cannot teach or suggest, in particular, an outlet centrally disposed in a portion of the cross-flow chamber opposite the filter surface, as presently claimed.

Raehse and Harm are cited by the Examiner as teaching cylindrical filters for use in cross-flow filtration. The teachings of cylindrical filters when considered alone or in any combination with Brody or Castino do not disclose or suggest the device or methods of the pending claims. In particular, there is no disclosure or suggestion of an outlet centrally disposed in a portion of the *cross-flow chamber* opposite the filter surface, as presently claimed. As such, the element missing from Brody is not provided by either Raehse and/or Harm.

For the reasons set forth above, Applicants respectfully request that the Examiner reconsider and withdraw the rejection of claims 6-10, 26, 30, 40-49 and 60 under 35 U.S.C. § 103(a) as being unpatentable over Brody (*supra*) in view of Castino (*supra*) as applied to claims 1-10 above, and further in view of Raehse *et al.* (US 4,751,003) or Harm (US 4,722,902).

Claims 14-22, 34-39, 49-59 and 61-69 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Brody (*supra*) in view of Castino (*supra*), and further in view of Kopf (U.S. Patent No. 6,214,221) and/or Yamanishi *et al.* (US 2003/0134416 or U.S. Patent No. 6,949,355, herein referred to as "Yamanishi").

According to the Examiner, these claims differ from Brody in additionally requiring various means and method steps to enhance culturing of and growth of the concentrated leukocytes or substances being derived therefrom, including beads, cell adhering substrate and screen, tissue culture vessel to receive mature cell cultures, separated mature from immature cell cultures, temperature control means, and wash and drain lines. The Examiner alleges that Castino teaches constant recirculation of fluid between crossflow membrane and

recovery unit. The Examiner further alleges that Yamanishi teaches such cell culture media substrates and cell culturing and maturing method steps and system components. The Examiner further believes that Yamanishi teaches use of beads, washing and draining means, antigen/antibody binding substances and substrates, culture of stem cells, separation of mature from immature cells. According to the Examiner, Kopf teaches usual cell culture media substrates and cell culturing and maturing method steps and system components, washing and draining means, antigen/antibody binding substances and substrates, culture of stem cells, separation of mature from immature cells, as well as the use of beads, separation of mature from immature cells, and temperature control. The Examiner believes that it would have been obvious to have augmented the crossflow filtration loop and recycling system and method of Brody in view of Castino, with the various means to culture leukocyte-derived substances, cells, and stem cells, as suggested by Yaminishi or Kopf. According to the Examiner, this combination would enrich leukocyte-product cell populations to promote growth and maturing of cell cultures, so as to have a complete cell growth and culturing system in one convenient and central location and avoid loss of cell populations and leukocyte ingredients that would otherwise result in transport of cell cultures between processing facilities.

Applicants respectfully disagree with the rejection and reasoning and submit that as explained above Brody fails to teach or suggest multiple elements of the present claims. Brody does not teach an outlet centrally disposed in a portion of the *cross-flow chamber* opposite the filter surface, as presently claimed. Instead, as shown in Fig. 1, Brody teaches a microfilter with an outlet (or, feed exit (2)) that runs parallel to the barrier channel (depicted in Fig. 2) and is disposed at the end of a feed flow channel, which is configured to extend past the barrier channel region. As above, the outlet (7) cited by the Examiner is in the filtration chamber and not in the cross-flow chamber as recited in the pending claims. Also, while Brody does mention red and white blood cells, the filtration process described is directed toward collecting cell-free plasma by filtering out particulates in blood. The reference provides no teaching or suggesting on how to separate or enrich for any cell in the blood; all of the blood constituents except for plasma are sent to the feed exit. In contrast, the presently claimed device enriches the sample of blood

constituents for leukocytes, *i.e.*, leukocytes are enriched over other particulates because red blood cells and plasma, for example, are selectively removed from the sample. Brody fails to teach or suggest this aspect of the pending claims.

Castino fails to provide the missing elements of Brody. Applicants, as above, respectfully direct the Examiner's attention to Applicants' previous arguments, *e.g.*, on pages 14-15 in the response filed on December 28, 2007, which points out why Castino fails to render the claims obvious. In addition, while Castino uses cross-flow membrane filtration, the reference is directed to extracting cell produced antiviral substances (CPAS), which can be, for example, an interferon or other biomolecule that is in the weight ranges of tens to hundreds of thousands Dalton. As the reference indicates, leukocytes can be used to produce the cell produced antiviral substance, but the leukocytes are not of interest with respect to the filtration process or device; they merely serve as producers. Therefore, while Castino may teach enrichment of a cell produced antiviral substance, the reference fails to teach the enrichment of any cell, much less the *enrichment of leukocytes.* Furthermore, Castino provides no schematic configuration of a filtration apparatus; thus, it cannot teach or suggest a device as presently claimed.

Kopf also fails to teach or suggest the missing elements of Brody. Kopf is directed to "purifying target biological substances, such as selected proteins, antibodies, antigens..." *See* column 1, lines 9-10. Kopf does not even mention leukocytes; thus, the reference cannot teach or suggest *enrichment of leukocytes.* Also, as shown in Figures 5 and 6, Kopf (like Brody) only teaches outlets from the cross-flow filter module that are disposed in an orientation parallel to the filter surface and located outside of the cross-flow chamber. The Examiner has cited Kopf as teaching cell culture media, substrates and cell culturing methods and steps. These media, substrates and cell culture methods when considered alone or in any combination with Brody, Castino, and/or Yamanishi do not disclose or suggest any device or method currently claimed. Therefore, the references fail to provide any teaching or suggestion whatsoever for an outlet centrally disposed in a portion of the cross-flow chamber opposite the filter surface and the use of such a device to produce a cell population enhanced in leukocytes, as presently claimed.

Finally, Yamanishi fails to teach or suggest the elements missing from Brody. While Yamanishi, like Kopf, may disclose cell culture media substrates and cell culturing and maturing method steps and system components. These concepts when consider alone or in combination with Brody, Castino, and/or Kopf do not disclose or suggest a device as presently claimed. Further, the Examiner believes that Yamanishi teaches the use of beads, washing and draining means, antigen/antibody binding substances and substrates, culture of stem cells, separation of mature from immature cells. Again, when considered alone or in any combination with Brody, Castino and/or Kopf none of the devices or methods teach or suggest an outlet centrally disposed in a portion of the cross-flow chamber opposite the filter surface, as presently claimed. As the reference does not even teach cross-flow filtration it cannot provide any teaching or suggestion regarding the positioning of an outlet for a cross-flow chamber. Also, Yamanishi cannot provide any teaching or suggestion for using the presently claimed tangential flow filtration device to prepare a cell population enriched for leukocytes since it does not even teach tangential flow filtration.

Applicants respectfully request that the Examiner reconsider and withdraw the rejections of claims 14-22, 34-39, 49-59 and 61-69 under 35 U.S.C. § 103(a) as being unpatentable over Brody (*supra*) in view of Castino (*supra*), and further in view of Kopf (U.S. Patent No. 6,214,221) and/or Yamanishi *et al.*

Claims 61-69 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Brody (*supra*) in view of Castino (*supra*) as applied to claims 1-10, 12, 14, 33, 40-48 and 60 above, and further in view of Yamanishi (*supra*). The Examiner submits that these claims differ in additionally requiring various means and method steps to enhance culturing and growth of the concentrated leukocytes or substances being derived therefrom, including beads, a cell-adhering substrate and screen, a tissue culture vessel to receive mature cells cultures, separation of mature from immature cell cultures, a temperature control means, and wash and drain lines. According to the Examiner, Yamanishi teaches such cell culture media substrates and cell culturing and maturing method steps and system components. The Examiner further alleges that Yamanishi teaches use of beads, washing and draining means, antigen/antibody binding substances and

substrates, culture of stem cells, separation of mature from immature cells. The Examiner believes that it would have been obvious to have augmented the crossflow filtration loop and recycling system and method of Brody in view of Castino, with the various means to culture leukocyte-derived substances and cells and stem cells and recirculate and return cell populations, as suggested by Castino and further suggested by Yaminishi. Further, the Examiner alleges that Castino and Yaminishi further would be used to both enrich leukocyte-product cell populations and promote growth and maturing of cell cultures, so as to have a complete cell growth and culturing system in one convenient and central location and to avoid loss of cell populations and leukocyte ingredients that would otherwise result from transport of cell cultures between processing facilities.

Again, Applicants respectfully disagree to the Examiner's rejection and the reasoning to support such a rejection. Brody and the other cited references either taken alone or combined fail to teach or suggest the present claims. In particular, Brody, the primary reference, does not teach an outlet centrally disposed in a portion of the *cross-flow chamber* opposite the filter surface, as presently claimed. Instead, as shown in Fig. 1, Brody teaches a microfilter with an outlet (or, feed exit (2)) that runs parallel to the barrier channel (depicted in Fig. 2) and is disposed at the end of a feed flow channel, which is configured to extend past the barrier channel region. The outlet (7) cited by the Examiner as being disposed in the cross-flow chamber is instead in the filtrate chamber. Also, while Brody does mention red and white blood cells, the filtration is directed towards collecting cell-free plasma by filtering out particulates in blood. The reference provides no teaching or suggestion as to how to separate or enrich for other particulates in the blood; all of the constituents except for plasma are sent to the feed exit. In contrast, the presently claimed device enriches the sample of blood constituents for leukocytes, *i.e.*, leukocytes are enriched over other particulates because red blood cells and plasma, for example, are selectively removed from the sample. Brody fails to teach or suggest this aspect as the pending claims. As indicated above, Castino and Yamanishi fail to provide the missing elements of Brody. Castino is directed to enriching for a cell produced antiviral substance and not to enriching for a cell, such as a leukocyte. Also, the Castino does not even discuss a

separation device much less the positioning of an outlet for a cross-flow chamber. Thus, Castino cannot provide any teaching or suggestion of a tangential flow filtration device comprising an outlet in a portion of the cross-flow chamber *opposite the filter surface*, as presently claimed.

Finally, as above, Yamanishi does not teach or suggest cross-flow filtration; thus, it also cannot provide any teachings or suggestions on how to position an outlet for a cross-flow chamber. Furthermore, the reference does not provide any teaching or suggestion for using the presently claimed tangential flow filtration device to prepare a cell population *enriched for leukocytes*. Accordingly, Applicants submit that the cited references either taken alone or combined fail to teach or suggest each and every element of the present claims. Applicants respectfully request that the Examiner reconsider and withdraw the rejections of claims 61-69 under 35 U.S.C. § 103(a) as being unpatentable over Brody (*supra*) in view of Castino (*supra*) as applied to claims 1-10, 12, 14, 33, 40-48 and 60 above, and further in view of Yamanishi (*supra*).

Applicant's arguments in the previous response filed December 28, 2007 with respect to claims 1-69 were considered by the Examiner, but are considered largely moot in view of the new ground(s) of rejection. According to the Examiner, Brody much more clearly discloses tangential fluid through a cross-flow filtration membrane with a centrally disposed flow outlet, the filtration membrane having a pore size range overlapping all of the instantly recited pore size ranges and optional selective removal or retaining of blood or plasma sample/analytical cell populations depleted or enriched in leukocytes and erythrocytes.

As above, Applicants have reviewed Brody and must respectfully disagree with the Examiner's reasoning and rejections. Brody, as set forth above, fails to teach or suggest the present claims. Specifically, Brody, the primary reference for the Examiner's rejections, does not teach an outlet centrally disposed in a portion of the *cross-flow chamber* opposite the filter surface, as presently claimed. Instead, as shown in Fig. 1, Brody teaches a microfilter with an outlet (or, feed exit (2)) that runs parallel to the barrier channel (depicted in Fig. 2) and is disposed at the end of a feed flow channel, which is configured to extend past the barrier channel

region. The outlet (7) referred to by the Examiner is not in the cross-flow chamber, but is in the filtrate chamber. As such, the outlet (2) in Brody that is the cross-flow chamber is not and does not suggest an outlet that is centrally disposed in a portion of the cross-flow chamber opposite the filter surface.

Also, while Brody does mention red and white blood cells, the filtration device and methods of Brody are directed toward collecting cell-free plasma by filtering out particulates in blood. The reference provides no teaching or suggestion regarding how to separate or enrich the other particulates in the blood; all of the constituents except for plasma are sent to the feed exit. In contrast, the presently claimed device *enriches the sample of blood constituents for leukocytes*, i.e., leukocytes are enriched over other particulates because red blood cells and plasma, for example, are selectively removed from the sample. Brody fails to teach or suggest this aspect. At least for these reasons above, Brody cannot anticipate the present claims. Furthermore, the cited references fail to provide these missing elements. Therefore, none of the present claims are rendered obvious.

As above, the Examiner is respectfully requested to reconsider and withdraw the rejections of the pending claims 1-69 in view of the above amendments and remarks.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 206-467-9600.

Respectfully submitted,

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